Green Bioprocessing of Algae-Derived Biopolymers

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Algae-based biopolymers may be modified by adding additives, plasticizers, and compatibilizers to enhance the intermolecular force of contact between components, and boost material strength, flexibility, and durability. Moreover, biopolymers are widely used in cosmetics, medicines, and food packaging. Furthermore, algal biopolymer could be used as a food additive due to its high nutritional content.

Keywords: microalgae ; value-added products ; biochar ; biopolymer

1. Introduction

In comparison to petroleum-based synthetic polymers, microalgae-driven biopolymers are thought to be the most sustainable biomass feedstock for the synthesis of biopolymers in the direction of a global circular bioeconomy ^[1]. Recent research reports that when compared to petroleum-based polymers, algae-based biopolymers have better mechanical properties ^[2]. Additionally, algae-based biopolymers may be modified by adding additives, plasticizers, and compatibilizers to enhance the intermolecular force of contact between components, and boost material strength, flexibility, and durability ^{[3][4]}. Moreover, biopolymers are widely used in cosmetics, medicines, and food packaging. Furthermore, algal biopolymer could be used as a food additive due to its high nutritional content ^[5]. Utilizing innovative biopolymers such as chitosan dramatically improves processes such as medication delivery and tissue regeneration ^[6]. Biopolymers and their composites are intensively used in contemporary technologies such as 3D printing ^{[2][8]}.

2. Biopolymers

2.1. Production of Biopolymers from Algae Biomass

Algae can grow everywhere, from marine environments to lichens to freshwater springs, displaying heterotrophic, mixotrophic, and autotrophic lifestyles. The growth of algae can be controlled in large-scale cultivation to increase the yield of lipids, hydrocarbons, and polysaccharides ^[9]. To produce biopolymer from algae, two linked stages are required. In the first stage, the algae are cultivated under high concentrations of nitrogen and nutrients to reach high cell densities. After reaching this point, some algae are transferred to the second stage, where salinity and nitrogen starvation are applied ^[10]. At this point, the biopolymer production phase is started by algae cells ^{[8][11]}. Many methods, such as solvent extraction, microwave-assisted extraction, ultrasound-assisted extraction, and supercritical water extraction, are used to extract polymers from algae biomass.

2.1.1. Solvent Extraction

The use of a solvent for biopolymer extraction is an easy method; however, the application of this method requires vast quantity of chemicals. In addition, compared to fermentation, solvent extraction is simpler and requires less downstream processing. Solvent extraction includes chemical agents added to and mixed with algal biomass to produce polymer precipitates ^[12]. To improve the accumulation process of biopolymers, optimization of all parameters (physical and chemical) is needed. Faidi et al. ^[13] used mineral acids (pH of 1.5) to extract alginate biopolymer from *Padina pavonica* algae. The authors reported that operation conditions such as centrifugation, sifting, and filtration significantly affected the extraction process. However, to reduce the effect of mechanical operation conditions, screening of productive algae is needed. For example, Morales-Jiménez et al. ^[14] investigated the biopolymer productivity of six algae strains, *Porphridiun purpureum*, *Synechocystis* sp., and *Nostoc* sp. which produced high biopolymer yields of 83, 204, and 323 mg/L, respectively. To conclude, biopolymer solvent extraction is a simple and easy method; however, the main disadvantage is the use of chemical solvents in huge quantities.

2.1.2. Microwave Assisted Extraction

Microwave-assisted extraction is a green and novel method to extract value-added products from algae biomass. The biopolymer inside an algae cell can be extracted using a microwave-assisted extraction approach. The advantages of microwave extraction are compactness, a quick and uniform process, low consumption of solvents, short experiment times, and no requirement of energy ^{[15][16]}. In a study, microwave-assisted water extraction was used to extract high yields from *Mastocarpus stellatus* red algae. The study reported that a 6 min operation time and a temperature of 150 °C were the optimum conditions ^[16]. In addition, the strength of biopolymers can be promoted when the temperature is increased. Microwave-assisted extraction is suitable for industrial applications as syneresis of biopolymeric gels is avoidable, and one can investigate the use of electromagnetic waves to aid in the manufacturing of biopolymers and create new methods to reach high yield and cost-effectiveness. However, because the microwave extraction method uses a small quintile of solvent, optimizing operating conditions remains a challenge that requires further investigation.

2.1.3. Ultrasound Assisted Extraction

Ultrasound waves cause cavitation, which causes turbulence and causes agitation and collisions in microparticles found in algae biomass. Ultrasound energy produces vibrant energy, which disrupts the cell walls of algae, improving the transfer rate and the removal of biopolymers $^{[1Z]}$. Compared to conventional methods, the advantages of ultrasound-assisted extraction include decreasing extraction time from hours to minutes and using room temperature to process the extraction. Extra separation methods such as membrane separation could be used, which are considered environmentally friendly and could reduce the material losses $^{[18]}$. Moreover, the biopolymer yield extracted by ultrasound was 33% higher than the conventional method. Flórez-Fernández et al. $^{[19]}$ reported that the ultrasound extraction method minimized the extraction time four times when alginate was isolated from *Sargassum muticum*. Furthermore, the authors found that temperature, ultrasound frequency, and sonication time were the main parameters that affected all processes. The authors reported that an increase in sonication time from 5 min to 30 min resulted in 10% more yield (from 5% to 10%). Although the ultrasound extraction method saves time and energy, much development is needed to increase the yield of biopolymers.

2.1.4. Subcritical Water Extraction

Subcritical water extraction is a promising extraction technology used to remove a value-added product from algae biomass. In this process, the water is heated above boiling temperature (up to 373 °C) and pressurized under the critical pressure point (221.2 bar) ^[20]. Compared to conventional extraction methods, supercritical extraction has many advantages, such as the use of water as the solvent, which eliminates the use of harmful chemicals and results in high yield and quality, low energy consumption, and a short reaction time ^[21]. In a study, Saravana et al. ^[22] used supercritical water extraction to extract fucoidan biopolymer from *Saccharina japonica* algae. The authors reported that using supercritical water extraction resulted in 4.85% of fucoidan being removed from algae biomass while conventional methods removed 2.47%. Alboofetileh et al. ^[21] reported that under optimal conditions of 29 min extraction time and 150 °C temperature, fucoidan removal efficiency from *Nizamuddinia zanardinii* was 25.98%. Likewise, Saravana et al. ^[23] examined the performance of supercritical water extraction combined with a deep eutectic solvent for the removal of fucoidan and alginate biopolymers from *Saccharina japonica* algae. The results reported that high removal efficiencies for alginate (28.1%) and fucoidan (14.93%) can be achieved using supercritical water extraction. More investigation is required to optimize the operation conditions, such as temperature and pressure.

2.2. Biopolymers Produced from Algae Biomass

2.2.1. Poly Hydroxy Alkanoate (PHA)

PHA produced by microorganisms is environmentally friendly and resembles petrochemical polymers in terms of its characteristics ^[24]. Stress brought on by a nitrogen deficiency can encourage the development of biopolymers. Many microalgae strains such as *Synechococcus subsalsus* and *Spirulina* were able to develop novel 14–18 carbon chain PHA biopolymers, but *Chlorella minutissima* was unable to do so, even in the absence of nitrogen. PHA monomer composition is strongly influenced by microbial strains and culture strains ^[25]. The optimization of algal PHA production is an essential step; thus, more bioprocess lab-scale studies are required to upscale all processes. Ramos et al. ^[26] suggested a mixed-integer nonlinear programming model. The authors chose the best circumstances that are suitable for the plant and maximized its net present value. Additionally, this can be useful in deciding between several options for extracting biopolymers from cells and choosing the best strategy to extract the highest quantity of biopolymers.

2.2.2. Poly Hydroxy Butyrate (PHB)

PHB, a polar and optically active biodegradable polymer, has received a great deal of interest because of PHB similarities to polypropylene in terms of physical and chemical characteristics ^[27]. In comparison to lignocellulosic biomasses, the presence of high starch content promotes the synthesis of PHB in the highest quantities ^[28]. Only a small amount of

material has been published that uses algae as a precursor in the creation of PHB, as the synthesis of PHB requires a significant amount of lipid ^[29]. Lipids are often accumulated in large amounts for the formation of biopolymers when cell growth rates are low. Cassuriaga et al. ^[24] investigated the impact of several factors on *chlorella fusca* PHB production. Their investigation yielded the highest level of PHB (17.4%), exceeding that produced by *Botryococcus braunii*, as reported by Kavitha et al. ^[30]. Recently, the possibility of producing PHB from agricultural runoff was investigated. Despite the modest PHB concentrations found, bacterial PHB production has a large potential ^[27].

2.2.3. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)

PHBV is produced by introducing 3-hydroxyvalerate, which decreases the crystallite nature of PHB^[31]. The use of PHBV in medicine, especially, in drug delivery systems has been reported. The unique PHBV physiochemical properties and slow rate degradation make them as good candidates for drug delivery systems. Many recent studies reported producing nano-structural systems using PHBV derived from alga. For instance, in the absence of a precursor, the PHBV production from glucose can be enhanced using the *Recombinant Bacillus megaterium* strain ^[32]. In the same study, more than 80% of PHBV was produced using the fed-batch method, whereas the batch approach produced just 46% of PHBV, revealing that fed-batch cultivation system is a promising technology for PHBV production from algae. These results showed the possibility of producing PHBV in large industrial scale from algal glucose.

2.2.4. Polylactide (PLA) and Polyalcohol

Polylactide and polyalcohol are polymers with wide applications in biomedical industries such as braces, sutures, bone screws, and bandages; thus, these biopolymers have received more attention in recent years. Many investigations have studied the composites of PLA produced from algae ^[33]. The biopolymer contents of algae can be modified to produce PLA biopolymers, which can be used in many medicine applications such as wound treatment, tissue regeneration, and tissue augmentation. Regarding polyalcohol produced from algae, they have many advantages such as water solubility, biodegradability, and high tensile strength. Bio-polyalcohol is generally used to produce biomaterials that have many applications. For example, one of the polyalcohol types is PVA that acts as a protective film, sizing agent, and emulsifier. In addition, bio-products made from PVAs can result in high-quality compositions. In a study, lipid-extracted algae were used to produce PVA, which was used to manufacture a bio-composites filter. The results showed that the introduction of PVA enhanced the thermal stability of the bio-composites by improving the mechanical characteristics ^[34].

2.2.5. Polysaccharides

Long-chain polysaccharides are used in the production of bio-materials due to their unique properties; these polymers are highly compatible with human systems as well as being biodegradable ^[35]. Algae can be used to produce polysaccharides including fucoidans, alginates, galectins, glucans, ulvans, carrageenan, and porphyrin. Cosmetics, tissue engineering, and cosmetic surgery are the main application of polysaccharides.

2.2.6. Alginate

Alginate is freely available in many *undaria pinnatifida* species (more than half). Alginate is used in many sectors such as drug delivery systems and tissue engineering. The introduction of alginates to produce hydrogels can enhance the stiffness, recoverability, and flexibility of these hydrogels. In addition, alginate has high adsorption capacity and absorption abilities ^[36]. Yuan and Macquarrie ^[37] examined the extraction of alginate from algae biomass by two methods: extraction and a bio-refining process. the results showed that the extraction method produced alginate of 23.13% more than the biorefinery method. Nevertheless, the biorefining approach generally produces two different types of alginates that differ in characteristics ^[38]. Moreover, pH is a significant factor affecting the alginate extraction process from algae. Alginate extraction under acidic pH produced insoluble alginic acids that adversely affect the alginate extraction. On the other hand, alkaline pH environment could produce high alginate yields ^[39].

2.2.7. Fucoidan

Fucoidan is widely used in cancer therapy, health products, and medicines. Brown algae is rich in Fucoidan, which has a heterogeneous structure. Fucoidan's structure can be determined in part by the source and extraction technique. Fucoidans have a high level of anticancer action at low molecular weights and high sulfur concentrations $^{[40]}$. High-molecular-weight fucoidan is used in nanomedicine and medication delivery and can be produced by non-degrading extraction $^{[41]}$. Fucoidan produced from *F. evanescens* has valuable properties such as a high monosaccharide content (96.1%), a low molecular weight (188 kDa), excellent anticomplement activity, and a high degree of sulfation (0.5). Moreover, fucoidan has high neutrophil transmigration inhibition (93%), significant anticancer efficacy, and equivalent antitumor effects compared to the conventional approach $^{[18]}$.

2.2.8. Laminarin

Laminarin is a non-hydrocolloid polysaccharide that includes long chains of glucose ^[42]. In terms of Laminarin structure, the predominance of single chain and low polydispersity are the main characteristics. Laminarin is consumed in the ethanol production process, marine carbon cycle, and medication administration ^[43]. Oxidation-reduction reactions enhance the performance of anti-inflammatory, antioxidants, and antitumor. In the Rajauria et al. ^[42] study, the authors increased laminarin content by purification. The laminarin has high anisometric stretching intensity and high antioxidant activity. Seaweed algae extract contains low-molecular-weight laminarin, which can be separated by using the membrane process ^[39].

2.2.9. Carrageenan

Carrageenan Red seaweed is often used to extract the polysaccharide carrageenan. It is used in medication delivery systems for the prevention of membrane fouling ^[44]. Using an ultrasonic pre-treatment at 90 °C for 15 min, carrageenan was extracted from *K. alvarezii* with a 56% extraction yield. The extraction technique affects the yield of the carrageenan. The molecular weights of carrageenan are decreased and dispersed because of extraction $^{[45]}$. The k-Carrageenan has a high viscosity and strong gel strength. While viscosity is only reliant on the extraction temperature, the gel strength of k-carrageenan depends on its monosaccharide concentration, purity, and critical gel temperature $^{[46]}$. **Table 1** shows different methods for biopolymer extraction from microalgae.

Microalgae Species	Isolation Method	Solvent	Isolation Conditions	Biopolymer	Yield	Reference
Alaria esculenta, Saccharina latissima and Ascophyllum nodosum	Solvent extraction	Water	0.2 M HCl and 0.1 M NaHCO ₃	Alginate	 Alaria (10%) Saccharina (15%) 	[47]
Ulva sp.	Solvent extraction	Dimethyl Sulfoxide	 Temperature 180 °C, Residence time 40 min. 	РНА	77.88%	[48]
Nizamuddinia zanardinii	Subcritical water extraction.	Water	 Temperature 150 °C, Residence time 29 min. 	Fucoidan	25.98%	[21]
Saccharica japonica	Subcritical water extraction.	Water	 Temperature 127 °C, Residence time 12 min. 	Fucoidan	13.65%	[22]

Table 1. Isolation techniques for biopolymer extraction from microalgae.

2.3. Bio-Composite Polymers

Biopolymers are often made using techniques such as electrospinning, melt casting, etc. Because of their low cost and low risk of tissue damage, algae–polymer composites are a viable option in biomedical industries ^[33]. The potential of biocomposite materials (also known as "green composites") to replace traditional materials used in manufacturing industries has substantially enhanced their attractiveness recently. Many researchers have been drawn to bio-composites because of their advantages over typical synthetic materials, including their ability to be composited after their expiration date, their ease of disposal, and their ability to be sustainable and renewable. Furthermore, bio-composites can be applied to a wide range of items due to their similar mechanical qualities. Cinar et al. ^[3] provided detailed descriptions of how to create bio-composite polymers in their literature, along with illustrations of how to characterize them. However, there have been improvements in the synthesis of composite algal biopolymers ^[3]. In another study, Kumar et al. ^[35] reported a new method to blend aqueous extracts of algae and alginate. Sayin et al. ^[33] reported that dried algae powders were combined with PLA and hot-pressed at 180 °C to create biopolymer composites, while Tran et al. ^[34] created lipid-extracted algae-PVA composites using the ultrasonication method. Important criteria for the manufacturing of composite materials include particle size and distribution as well as algal filler shape. Due to the intense interfacial contact, ultrasonic treatment disturbs and produces smaller algae particles that are readily reinforced. For the manufacture of algal bio-composites blends, one can employ physically assisted procedures such as microwave-assisted, supercritical-fluid-assisted, UV-assisted, etc.

2.4. Applications of Algae Biomass-Based Biopolymers

There are many applications of algae biomass-based biopolymers in the literature. The significant use of algae-based biopolymers is discussed in this section. **Figure 1** shows the possible applications of algal-driven biopolymers.



Figure 1. Applications of algal-driven biopolymers.

2.4.1. Biosensor

Recent technical developments have facilitated the development of sophisticated electrochemical biosensor structures, which are essential for healthcare monitoring ^[49]. Two of the most studied polysaccharides in this area are chitosan and carboxymethyl cellulose due to their high qualities, which include biocompatibility, biodegradability, non-toxicity, naturally renewable, and the capacity to create adherent thin films ^[50]. By using parallel reactions that can happen in enzymatic reactions with substrate or product, laccase-based biosensors can be used to determine medications in a non-direct manner ^[51].

2.4.2. Removal of Pollutants

Biopolymers include starch, cellulose, polysaccharides, alginate, and chitosan, which are naturally occurring biopolymers. These biopolymers adsorb polycyclic aromatic hydrocarbons, metals, sulfide-containing contaminants, benzene, and other pollutants, creating a complex hybrid while removing contaminants from wastewater [52]. The use of biopolymers for the adsorption process results in strong and efficient adsorption and may not result in secondary pollution or harmful byproducts. The ability to restore and reuse it is also simple [53]. Clays and biopolymers cooperate in an adsorption process to remove dyes and remediate heavy-metal contamination. The removal of hydrophobic contaminants requires the use of biopolymers such as polysaccharides and polypeptides because natural clays are inadequate for this purpose. This results in a powerful combination that may be used for environmental cleanup ^[54]. When clay and biopolymer are combined as bio-composites, compared to when they are used individually, their qualities, such as resilience to low wettability, pH fluctuations, and poor specificity, are significantly improved. According to Xia et al. [55], two bacterial strains from wastewater treatment plants (WWTPs) Klebsiella sp. (designated as EPS-K) and Bacillus sp. (EPS-B) were used to remove mercury. For the elimination of organic pollutants, a biopolymer with integrated phosphate groups (PCel) has been developed. Using phosphoric acid and sodium tripolyphosphate to modify cellulose surface has been investigated to produce biopolymers. The highest levels of adsorption for investigated organic pollutants were at acidic-to-neutral pH, with a capacity of 47.58 mg/g for Rhodamine B and 45.52 mg/g for Amitriptyline [55]. Several investigations used modified cellulose for adsorption as its surface has hydroxyl (OH) groups [56].

2.4.3. Biomedical Applications

Biopolymer possess valuable properties such as non-toxicity, biodegradability, water-holding capacity, and high tensile strength. These unique properties make biopolymers a good feedstock for the biomedical engineering sector, especially, in bone-tissue engineering and regenerative medicines. According to a recent study by Sathiyavimal et al. ^[52], since hydroxyapatite (HAp) is a crucial mineral for human bone, chitosan can be used to create a composite biopolymer of HAp. Bahmani et al. ^[58] employed soybean oil epoxidized acetate (SOEA) in combination with HAp nanoparticles, comparing the mechanical characteristics of the biopolymer after adding hydroxyethyl acrylate to one portion of the composite and removing it from another. Sayin et al. ^[33] investigated marine alga–PLA composites for collagen membranes. Polylactide was used in this study to improve the qualities of algal strains such *Galaxaura oblongata, Corallina elongate, Sargassum vulgare, Cystoseria compressa* and *Stypopodium schimperi*. Regarding skin-grafting applications, type IV MAP (*Sargassum vulgare*) showed the greatest qualities.

2.4.4. 3D Printing

Due to 3D printing's ability to build complicated structures quickly and accurately, it has gained popularity recently. The challenge currently is to describe the mechanical and biological characteristics of naturally existing bio-based materials ^[59]. Ponthier et al. ^[16] filled a PVA bio-composite for 3D printing with the algae *Nanochloropsis salina*. Because of their adaptability, biopolymers can be used for a variety of purposes that require different material characteristics. 3D-printed biopolymers are employed in adsorption for environmental remediation and medicinal applications. To manufacture fillers for the methyl orange degradation, Sangiorgi et al. ^[60] used polylactic acid that had been treated with TiO₂. Because the composite contains 30% TiO₂, 100% of the methyl orange may be completely degraded in 24 h. The stem cell activity of 3D-printed scaffolds coated with nanoscale ceramics was investigated ^[61].

2.4.5. Antioxidant

In the food business, biopolymers are mostly used as antioxidants. Compared to other antioxidants, biopolymers are preferable since they are non-toxic. Biopolymers do not show the negative health impacts that manufactured antioxidants do ^[57]. For example, biopolymers prevent the oxidation of unsaturated lipids at the oil–water interface that keeps food fresh ^[62]. Pérez Córdoba and Sobral ^[63] investigated the antioxidant characteristics of three different gelatin biocomposites: gelatin–sodium caseinate (G-C), pure gelatin (G), and gelatin–chitosan (G-Ch). The results showed that G–C compounds combined with actives showed high antioxidant properties. By utilizing active enzymes such as *Aspergillus oryzae* and *Aspergillus flavipes*, Zanutto-Elgui et al. ^[64] used goat and cow milk to create bioactive peptides. Studies have shown that this process has a potent antioxidation ability of up to 92.5% DPPH equivalent, making it valuable in the food and pharmaceutical industries. Gopu and Selvam ^[65] extracted *Amphiroa rigida*, an algae strain, using ultrasound to produce a powerful antioxidant. Given that the developed ARPS can scavenge DPPH and ABTS, it was determined that it also has strong antioxidant effects.

2.5. Degradation of Bioplastics

Prior to now, the biodegradability of polymers was assessed using tests for microbial development, tensile strength changes, and the loss of other physical characteristics that fall within qualitative evaluation. Soil burial tests were used as one of the approaches for determining how well the bio-composites were degrading, even though quantitative assessment tests also include analytical procedures for each reactant and product $\frac{[66]}{1}$. To reduce the quantity of CO₂ that the soil produced compared to the bio-composites, the soil was replaced with hydroscopic aluminum silicate [67]. In contrast to a non-biodegradable polymer matrix, which is disposed of by burning or landfilling, bio-composites with a biodegradable polymer matrix are disposed of via composting, which can be used as fertilizer. Because they are constructed of different components, it is challenging to recycle more complex bio-composites [68]. Biopolymers can degrade by a variety of chemical, biological, or even a combination of processes, including the four main types of degradation: thermal degradation, photodegradation, oxidative degradation, and high-energy degradation. Temperature, humidity, and the quantity and bacteria species affect the biodegradation rate. PLA was recycled as a monomer after hydrolysis and enzymatic breakdown. PLA degraded differently depending on the environment it was exposed to [69]. PLA film's permeability to oxygen and water vapor considerably increased as the number of extrusions was increased during processing ^[70]. Numerous studies have been conducted to determine how successfully certain polymers may be recycled. With the aid of catalysts, the thermal breakdown process of PHAs can produce its vinyl monomers [71]. Therefore, the threat posed by plastic and new plastic pollutants, including micro- and nano-plastics as well as plastic leachates, would be reduced because of the biodegradability of biopolymers and bio-composites. However, the product and its use determine the trade-off between strength, applicability, and degradability.

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